

## Leaching and Binding of Fumonisin in Soil Microcosms

LONNIE D. WILLIAMS,<sup>†,‡</sup> CHARLES W. BACON,<sup>‡</sup> FILMORE I. MEREDITH,<sup>‡</sup>  
ALAN J. FRANZLUEBBERS,<sup>§</sup> ROGER D. WYATT,<sup>†</sup> MARY A. SMITH,<sup>†</sup> AND  
RONALD T. RILEY<sup>\*,‡</sup>College of Agriculture and Environmental Sciences, University of Georgia, Athens, Georgia 30602,  
Toxicology and Mycotoxin Research Unit, USDA, ARS, Athens, Georgia 30604-5677, and  
Southern Piedmont Conservation Research Unit, USDA, ARS, Watkinsville, Georgia 30677-2373

Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is a water-soluble mycotoxin produced by *Fusarium verticillioides*. Our research objectives were to determine the leaching of FB<sub>1</sub> through soils and FB<sub>1</sub> binding in soil. Leachate columns were used to determine the movement of FB<sub>1</sub> through soil. FB<sub>1</sub>-contaminated corn screenings or water extracts containing FB<sub>1</sub> were placed on the surface of soil columns. In 100% sand columns, FB<sub>1</sub> leaching was only slightly retarded, whereas at 50%, 75%, and 100% Cecil sandy loam, approximately 60%, 50%, and 20% of the FB<sub>1</sub> was recovered in the column leachate, respectively. The FB<sub>1</sub> retained on the 100% Cecil sandy loam column was tightly bound. However, approximately 75% of the bound FB<sub>1</sub> was released with 5% formic acid and 5% formic acid/acetonitrile (1:1), indicating that the nature of the interaction was probably ionic. The results suggest that FB<sub>1</sub> is quite stable in soils and, while tightly bound, under certain environmental conditions could be released.

**KEYWORDS:** Fumonisin B<sub>1</sub>; Cecil sandy loam; *Fusarium verticillioides*; corn

## INTRODUCTION

Fumonisin is a mycotoxin produced by the fungus *Fusarium verticillioides* (syn = *F. moniliforme*). *F. verticillioides* parasitism of corn (*I*) has been extensively researched because this fungus produces a variety of chemically different mycotoxins on corn (2). Diseases of corn associated with *F. verticillioides* infection include seed rot, seedling blight, root rot, stalk rot, and kernel or ear rot (3–5). At present, at least 15 different fumonisins have been reported and other minor metabolites have been identified, although some of them do not occur naturally (6–8). Fumonisin B<sub>1</sub> (FB<sub>1</sub>) (Figure 1) is the most abundant of the naturally occurring fumonisins (6–8). Fumonisin B<sub>2</sub> and B<sub>3</sub> are also often found in *F. verticillioides*- and *F. proliferatum*-infected corn. The pure substances are amphipathic zwitterions which are water soluble and heat and light stable (9–11).

Animal diseases associated with FB<sub>1</sub> include equine leukoencephalomalacia, porcine pulmonary edema, and liver and kidney carcinogenesis in rodents. There have also been studies which suggest that FB<sub>1</sub> plays a role in increased incidences of esophageal cancer in humans (11).

Fumonisin of the B series are poorly absorbed, not metabolized, and rapidly excreted by animals, with only a small amount of the toxin being retained in the liver and kidney (11). Thus, most of the fumonisins consumed by farm animals are deposited

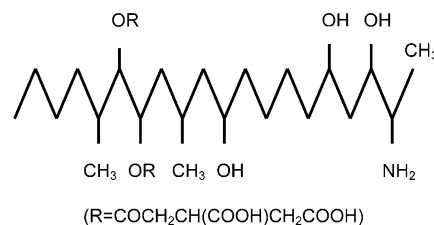


Figure 1. Chemical structure of fumonisin B<sub>1</sub>.

on the ground unmetabolized or as their hydrolyzed fumonisin metabolites (removal of one or both of the tricarballic acid side chains).

*F. verticillioides* and the fumonisins have become areas of concern for corn producers, processors, consumers, and regulators. The occurrence and contamination of corn is worldwide and can have health effects in animals and possibly humans (12). Recently, FB<sub>1</sub> was evaluated by the International Agency for Research on Cancer, and the evaluation was that there is sufficient evidence in experimental animals for the carcinogenicity of FB<sub>1</sub>. The overall evaluation was that FB<sub>1</sub> is possibly carcinogenic to humans (Group 2B) (12). In addition, little is known about the environmental fate of fumonisins, and therefore other routes of human exposure are possible (11). The Joint Expert Committee on Food Additives recommended to the Codex Committee on Food Additives and Contaminants a provisional maximum tolerable daily intake (PMTDI) for FB<sub>1</sub> of 2 µg/kg/body weight/day (13). The U.S. Food and Drug Administration final guidance for industry was issued in 2001

\* To whom correspondence should be addressed [telephone (706) 546-3377; fax (706) 546-3116; E-mail rriley@saa.ars.usda.gov].

<sup>†</sup> University of Georgia.

<sup>‡</sup> Toxicology and Mycotoxin Research Unit, USDA, ARS.

<sup>§</sup> Southern Piedmont Conservation Research Unit, USDA, ARS.

(<http://www.cfsan.fda.gov/~dms/fumongu2.html>). These recommendations are intended to minimize exposure from FB<sub>1</sub>-contaminated corn-based foods and feeds. However, since little is known about the environmental fate of fumonisins, other routes and sources of exposure could contribute to the total fumonisin intake by humans and animals. For this reason, the World Health Organization International Programme on Chemical Safety has recommended further research on the environmental fate of fumonisin (11).

Diseased corn parts infected with *F. verticillioides* that are not harvested could contribute to the surface-soil plant litter. It is possible that fumonisin levels in soils and water could be high, given that (1) fumonisins are highly water soluble and heat and light stable (9–11), (2) fumonisins can accumulate to high levels in moldy corn parts and plant debris, and (3) the majority of fumonisins are rapidly excreted intact by farm animals (11).

While the amount of fumonisin that enters the environment from plant debris is unknown, it is possible to estimate the amount of FB<sub>1</sub> that enters the environment from farm animal waste. Corn represents a large portion of the feed ration for many commercially produced livestock commodities. Livestock production plays an important role in the economy of the United States, so large amounts of corn and other grains are produced annually to support these enterprises. The total corn crop for 1998 was 9.76 billion bushels (250 000 000 metric tons) (14). Assuming that 1–2 kg of fumonisin is produced per metric ton of corn (1–2 ppm), the annual total of fumonisin produced in 1998 would have been 250–500 metric tons. Of the 250 000 000 metric tons of corn produced, 60% (150 000 000 metric tons) was used for animal feed, 20% was exported, and the remaining 20% was used to make cereal and other products (14). The 60% used for animal feed could have contained 150–300 tons of fumonisin contamination. Potentially 90% of the fumonisin consumed by livestock ends up in litter, in sewage, or on the ground each year (130–270 metric tons). There are limited data suggesting that soil microorganisms can metabolize fumonisins (11). However, little is known about the environmental fate of fumonisins after they are excreted from animals. In addition, a large amount of corn plant debris is left in the field or is plowed into the soil. Damaged corn and cob and stalk parts have all been shown to potentially contain high levels of fumonisin (15). In the only available published report on the fate of FB<sub>1</sub> in soil (16), it was found that when FB<sub>1</sub> was mixed with silty clay loam soil, it could not be recovered from the soil. It was concluded that FB<sub>1</sub> was either irreversibly bound or chemically altered in the soil (16). However, it is possible that other soil types do not interact as strongly with FB<sub>1</sub> or that there are conditions under which bound FB<sub>1</sub> can be released and become biologically available or enter the groundwater. If unaltered, fumonisins entering Cecil sandy loam soil (a common soil type in northeast Georgia) could potentially reach groundwater. The specific objectives of the research described herein were (1) to determine the leaching of FB<sub>1</sub> through Cecil sandy loam soils, (2) to determine if FB<sub>1</sub> is bound in this soil type, and (3) if bound or metabolized, then to determine the nature of the binding or metabolism.

## MATERIALS AND METHODS

**Fumonisin (Test Material).** Corn screenings naturally contaminated with FB<sub>1</sub>, or water extracts of this material, were used in studies to simulate leaching of FB<sub>1</sub> from corn debris through the soil, and subsequently into the ecosystem. The total amount of FB<sub>1</sub> present in the contaminated corn was determined by placing 1 g of finely ground

(1 mm screen in a Thomas Wiley mill), naturally contaminated corn screening in 125-mL Erlenmeyer flasks and adding 25 mL of 1:1 acetonitrile/water. The pH of the acetonitrile/water extracts was adjusted to 4.5 with 6 N HCl. These solutions were capped and placed on a rotary shaker for 6 h. After shaking, the extracts were filtered through Whatman No. 1 filter paper and analyzed by high-performance liquid chromatography (HPLC) or liquid chromatography/mass spectrometry (LCMS). Water was also used as the extraction solution, and this was compared to the FB<sub>1</sub> extraction efficiency of acetonitrile/water (1:1) in a time course study (15 min to 96 h). When miniature columns were used, the test material was water extracts of the corn screenings containing FB<sub>1</sub>; when intact soil cores were used (to be described later), FB<sub>1</sub>-contaminated corn screenings were placed directly on the surface of the soil, and water was allowed to percolate through the screenings, extracting the FB<sub>1</sub> and allowing it to enter the soil.

**HPLC Method.** Samples of the extracts (100  $\mu$ L) were combined with 500  $\mu$ L of *o*-phthalaldehyde (OPA, Sigma Chemical Co., St. Louis, MO) derivatizing reagent and 500  $\mu$ L of acetonitrile/water (1:1). Samples and reagents were maintained at 4 °C, and mixing of reagents and derivatization was accomplished at 4 °C using a Shimadzu model SIL-9A programmable autoinjector. The derivatized samples (50  $\mu$ L) were injected 3 min after mixing with the OPA reagent, and separation was accomplished using a Microsorb C18 column (3  $\mu$ m particle size, 4.6 mm i.d.  $\times$  5 cm length, Rainin Instrument Co., Woburn, MA), maintained at 27 °C with a mobile phase of methanol/1% phosphoric acid in water (66:34) and a flow rate of 0.8 mL/min. OPA-positive substances were detected using a Shimadzu RF-551 spectrofluorometric detector at 335 nm excitation and emission cutoff filter at 440 nm. Pure (>96%) FB<sub>1</sub> standard (17) was injected after every three unknown samples, and the FB<sub>1</sub> concentration in unknown samples was quantified on the basis of the areas under the peaks.

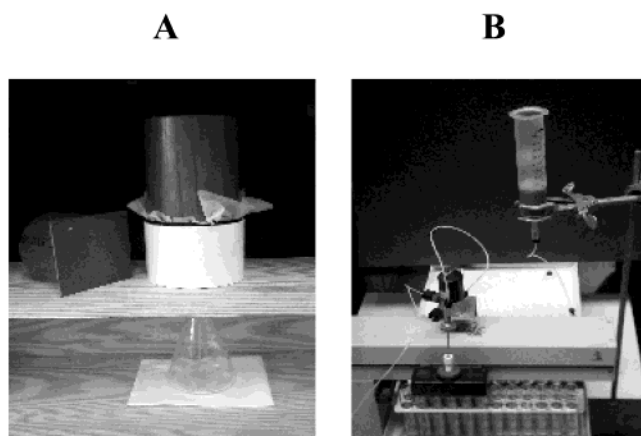
**LCMS Methods.** Soils or corn screenings were air-dried at room temperature, and then 1-g samples were removed and placed in 50-mL conical tubes with 30 mL of pure water for extracting corn screenings and 30 mL of 5% formic acid for extracting soils. The mixtures were shaken on a rotary shaker for 3 h. Samples were then centrifuged at 240 relative centrifugal force (rcf) for 30 min, and a 1-mL sample was removed and centrifuged at 20 000 rcf for 10 min. A 5–10- $\mu$ L aliquot was injected directly onto the LC at room temperature. Fumonisin were chromatographically separated on a Thermal Separations HPLC (Riviera Beach, FL), consisting of a model P2000 solvent delivery system and an AS3000 autosampler. Separations were done using an Intersil 5 $\mu$  ODS-3 column (150  $\times$  3 mm, Metachem Technologies, Inc., Torrance, CA). The flow was 0.2 mL/min, and the mobile phase was a 20-min gradient starting at 60% methanol/40% water (both containing 0.3% acetic acid) and ending at 90% methanol/10% water (both containing 0.3% acetic acid), followed with a 15-min re-equilibration with 60% methanol/40% water (both containing 0.3% acetic acid). The total run time was 35 min. The column effluent was directly coupled to a ThermoFinnigan LCQ Duo ion trap mass spectrometer (MS) (Woodstock, GA). The MS was operated in the electrospray ionization (ESI) positive ion mode, with an inlet capillary temperature of 225 °C, and the sheath gas was nitrogen (20 arb). For MS/MS of FB<sub>1</sub>, the collision energy was 30%, the parent *m/z* was 722.3, and mass fragments were scanned from 195 to 800 *m/z* and compared to an authentic FB<sub>1</sub> standard. FB<sub>2</sub> and FB<sub>3</sub> were also analyzed using single-ion monitoring (SIM), with the parent *m/z* of 706.3 for FB<sub>2</sub> and FB<sub>3</sub>.

**Test Soils Used in Leachate Columns.** Two different soils and various mixtures of the two soils were chosen for study. Washed fine sand was chosen to model the simplest soil system and contained very little organic material or mineral nutrients (Table 1). A Cecil sandy loam (fine, kaolinitic, thermic Typic kanhapludult) was collected under Bermuda grass sod near Watkinsville, GA. Samples of Cecil sandy loam and washed sand were analyzed by the University of Georgia Soil Testing and Plant Analysis Laboratory (Athens, GA). Cecil sandy loam contains a mixture of silt, clay, and sand and a much higher content of organic material and mineral nutrients than the washed fine sand (Table 1). The Cecil sandy loam was used in the leachate columns either as intact cores (see Figure 2A) or after air-drying and grinding.

**Table 1.** Comparison of the Chemical and Physical Analysis of Washed Sand and Cecil Sandy Loam<sup>a</sup>

|                         | sand | Cecil sandy loam |
|-------------------------|------|------------------|
| CEC (mequiv/100 g)      | 0.2  | 5.6              |
| pH                      | 5.7  | 5.6              |
| organic matter (OM) (%) | 0.1  | 3.4              |
| Ca (mg/kg)              | 9.3  | 404.5            |
| K (mg/kg)               | 3.2  | 52               |
| Mg (mg/kg)              | 1.8  | 59.6             |
| Mn (mg/kg)              | 0.6  | 36.1             |
| P (mg/kg)               | 0.8  | 26               |
| Zn (mg/kg)              | 0.3  | 0.9              |
| % sand (2–0.05 mm)      | 98.0 | 72.0             |
| % silt (0.05–0.002 mm)  | 0.0  | 14.0             |
| % clay (>0.002 mm)      | 2.0  | 14.0             |

<sup>a</sup> Samples of Cecil sandy loam soil and washed sand were analyzed by The University of Georgia soil testing and plant analysis laboratory (Athens, GA).



**Figure 2.** Example of a leachate column for obtaining soil cores from the field (A) and a leachate column for testing homogeneous mixtures of soils (B). Briefly, column A is fabricated from a 10- × 20-cm piece of thick-walled PVC pipe with a bevel cut at the bottom to facilitate obtaining field soil cores. Field cores were obtained by forcing the columns down into the soil using approximately 2 tons of pressure. The columns were carefully removed by excavating around the base of the columns. The soil cores obtained in this manner were tightly adherent to the inside surface of the PVC columns. To simulate rainfall, water was added to the rain simulator (shown to the left) and allowed to drain into the PVC leachate column. The rain simulator is a flat piece of perforated PVC glued to a piece of PVC pipe. Column B (60-cm<sup>3</sup> syringe) was used in experiments where FB<sub>1</sub> was in water solution and was allowed to pass through homogeneous sand or sand/soil mixtures. Syringes containing glass microfiber filters were filled with either washed sand or the homogeneous mixture of sand and soil (30 g). The filled columns were placed in an elevated clamp attached to a ring stand, and tubing connected the columns to a fraction collector.

The washed fine sand soil was always used by pouring directly into the column and never obtained as a core.

**Miniature Leachate Columns, FB<sub>1</sub> Binding to Soil.** To determine if FB<sub>1</sub> interacts with soil constituents, miniature leachate columns (60-cm<sup>3</sup> syringes) (Figure 2B) were used with either washed sand alone or a homogeneous mixture of washed sand and Cecil sandy loam. Mixtures of 50%, 75%, and 100% Cecil sandy loam soil and washed sand were used. Water extracts of the FB<sub>1</sub>-contaminated corn test material were percolated through the miniature columns (*n* = 3). Fractions were collected (1 mL each) over a 24-h period and analyzed to determine if FB<sub>1</sub> interacted with the soil constituents so as to retard or otherwise affect the retention or recovery of FB<sub>1</sub> in the eluate from the leachate columns.

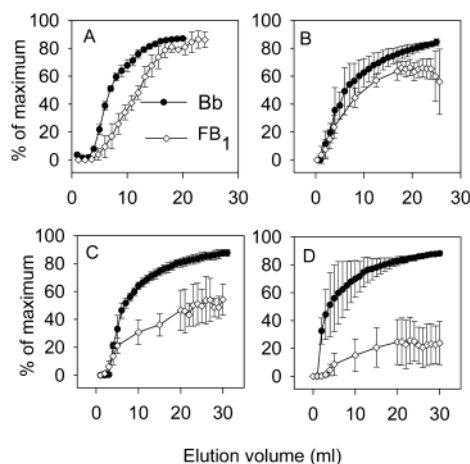
The FB<sub>1</sub> recovery, based on HPLC analysis, was compared to the movement of bromophenol blue (Bb), a dye that moved freely through sand columns and the sand and soil mixtures. A stock solution of Bb dye was prepared by mixing 0.5 g of Bb dye with 200 mL of water; the absorbance at 590 nm was approximately one. Aliquots (20–30 mL) of the dye solution were allowed to percolate through the small columns containing the previously described sand and sand/soil mixtures, and 1-mL fractions were collected over a 24-h period. The absorbance of these fractions was measured using a Beckman DU-65 spectrophotometer, and the values were compared to the absorbance of the stock dye solution. The absorbance values for the 1-mL fractions were greater than 80% of the maximum absorbance, which suggested that the dye had a relatively low affinity for the sand or sand/soil mixtures used in the miniature columns. The void volume was estimated by weighing 30-g samples of 100% sand, 50% sand/50% Cecil sandy loam, and 100% Cecil sandy loam, mixing the soil with 30 mL of water, centrifuging at 240 rcf for 60 min, and measuring the volume of the supernatant. The void volume was calculated on the basis of the water retained in the soil. The estimated void volumes of 100% sand, 50% sand/50% Cecil sandy loam, and 100% Cecil sandy loam were 7.5 ± 0.5, 10.5 ± 0.5, and 14.5 ± 0.5 mL (*n* = 6), respectively.

**Intact Soil Core Columns.** To determine the interaction of FB<sub>1</sub> with soils as they occur in the field, a model soil microcosm was constructed which consisted of cores (Figure 2A) of Cecil sandy loam soil. PVC columns filled with washed sand were also prepared and used in a similar manner. The cores were obtained from a local USDA field station in Watkinsville, GA. The soil core columns consisted of three 10- × 20-cm PVC columns of cored soils with fine nylon screens at the bottom. A funnel was attached to the bottom of the columns, which allowed the eluate to be collected into 500-mL Erlenmeyer flasks. Rainfall (2.4 cm<sup>3</sup>/cm<sup>2</sup>/day) was simulated using a 10- × 5-cm PVC column with a PVC base perforated with 1/16-in. holes in a 1-cm<sup>2</sup> grid pattern. Finely ground FB<sub>1</sub>-contaminated corn screenings were placed on the surfaces of the cores (50 g/core). The water was applied at hourly intervals for 10 h. The effluent from each application was collected and analyzed by HPLC for the presence of FB<sub>1</sub>. Leaching of FB<sub>1</sub> through soil cores was compared to leaching of FB<sub>1</sub> through PVC columns filled with washed sand.

To determine how tightly FB<sub>1</sub> binds to soil constituents, an acid displacement procedure was developed using mixtures of sand and Cecil sandy loam soil. The procedure consisted of mixtures (30 g) containing 100% sand, 50% sand/50% Cecil sandy loam soil, or 100% Cecil sandy loam, which were mixed in 50-mL culture tubes with FB<sub>1</sub> aqueous solutions containing either 188 or 33 µg of FB<sub>1</sub>/mL. The tubes were shaken for 12 h and centrifuged at 240 rcf, supernatants removed, and aliquots analyzed by HPLC for FB<sub>1</sub>. The soil samples were then consecutively extracted with acetonitrile/water (1:1), 5% formic acid, and acetonitrile/5% formic acid to determine if the FB<sub>1</sub> could be extracted with nonpolar or polar solvents. Formic acid was used because it was hypothesized that there might be a strong ionic interaction between FB<sub>1</sub> and the soil. Acetonitrile and water were added to each tube so as to attain a 1:1 mixture based on the calculated void volumes. The tubes were shaken and centrifuged, and FB<sub>1</sub> in the supernatant was determined by HPLC. Each sample was similarly extracted with 5% formic acid and then acetonitrile/5% formic acid. Selected samples from each soil extract were also analyzed by ion trap LCMS and LCMS/MS to confirm that the OPA-positive substances determined by HPLC, with the same mobility as FB<sub>1</sub>, were in fact FB<sub>1</sub> based on their mass and their fragmentation pattern compared to authentic standards of FB<sub>1</sub>. HPLC analysis of Cecil sandy loam extracted with acetonitrile/water (1:1) or 5% formic acid before adding FB<sub>1</sub> revealed no OPA-positive substances with mobility the same as that of pure FB<sub>1</sub> (data not shown).

**Statistical Analysis.** Statistical analysis was done using Sigma Stat software (Jandel Scientific, San Rafael, CA). One-way analysis of variance (ANOVA) was used, followed by tests for post hoc multiple comparisons. All data were expressed as mean ± standard deviation, and differences among means were considered significant if the probability was <0.05. For the time course study, the data were analyzed by nonlinear regression analysis and ANOVA.



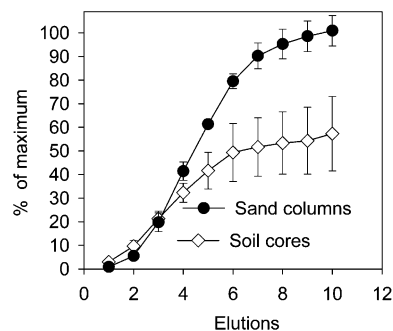


**Figure 3.** Comparison of the FB<sub>1</sub> concentration (◇) and the bromophenol blue (Bb) absorbance at 590 nm (●) in column leachate, expressed as a percent of the FB<sub>1</sub> concentration ( $123 \pm 4 \mu\text{g}$  of FB<sub>1</sub>/30 mL) or the Bb absorbance at 590 nm, respectively, of the solutions originally placed on the soil surface of the columns. Columns were loaded with washed sand (A) or homogenized mixtures of 50% (B), 75% (C), or 100% (D) Cecil sandy loam. FB<sub>1</sub> was water-extracted from 1 g of corn screenings, and Bb was dissolved directly into water and added on top of the sand or soil mixture. A total of 20–30, 1-mL fractions of the eluate were collected and analyzed for FB<sub>1</sub> or, in the case of Bb, absorbance at 590 nm, and these values were compared to the total FB<sub>1</sub> placed on the columns and the maximum absorbance of the stock dye solution. The results are expressed as the means  $\pm$  SD ( $n = 3$ ).

## RESULTS AND DISCUSSION

Fumonisin B<sub>1</sub> was rapidly and completely extracted from corn screenings using either pure water or acetonitrile/water (1:1) (data not shown). After 24 h of extraction with water, there was evidence of microbial growth, determined by microscopic examination and culture on potato dextrose agar (PDA) plates. One-way analysis of variance indicated that there were no significant differences among groups in either the water or acetonitrile/water extractions groups. However, linear regression analysis of the means with time indicated that there was a significant decrease of FB<sub>1</sub> content with time in the water-extracted sample ( $F = 7.9$ ,  $p = 0.009$ ,  $df = 26$ ). The rapid release and complete recovery of FB<sub>1</sub> by water extraction alleviated the need for extensive extraction periods, since maximum extraction of FB<sub>1</sub> was achieved within 1 h by either method.

Fumonisin B<sub>1</sub> moved rapidly through both simple and more complex soils intact. Elution of FB<sub>1</sub> through the miniature columns, applied to the surface of washed sand as water extracts of the corn screenings ( $123 \pm 4 \mu\text{g}$  of FB<sub>1</sub>/30 mL), was slightly retarded relative to the elution of Bb (Figure 3A). The calculated void volume of the miniature leachate columns filled with sand was approximately 7.5 mL. Thus, both the Bb and the FB<sub>1</sub> first appeared in the void volume but did not attain equilibrium until after the void volume had passed through the column. After 20 mL of the FB<sub>1</sub> solution had passed through the column, the concentration of FB<sub>1</sub> in the leachate was approaching the concentration that was in the original water extracts. The average cumulative total of FB<sub>1</sub> recovered in the leachate was  $98 \mu\text{g}$  of FB<sub>1</sub>, which was 80% of the total FB<sub>1</sub> calculated in 20 mL of water extract. Both Bb and FB<sub>1</sub> leaching exhibited a short initial lag phase, a rapid elution phase, and a slowly eluted terminal phase, kinetics consistent with a three-compartment model.

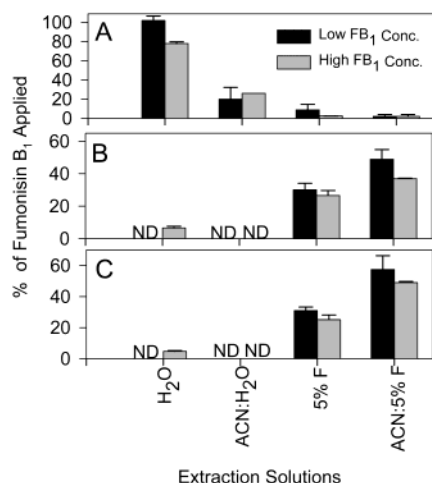


**Figure 4.** Elution of FB<sub>1</sub> from Cecil sandy loam field cores (◇) compared to FB<sub>1</sub> elution through columns filled with washed sand (●). Intact field cores of Cecil sandy loam soil were obtained as described in Figure 2, and 50-g samples of FB<sub>1</sub>-contaminated corn screenings were placed on the surface of both Cecil sandy loam and washed sand columns. Rainfall (10 days) was simulated by hourly application of 190 mL of water for 10 h, after which the collected eluates from the soil cores and sand columns were analyzed by HPLC for FB<sub>1</sub>. The values were expressed as a percentage (means  $\pm$  SD,  $n = 3$ ) of the total FB<sub>1</sub> applied to the columns.

Elution of FB<sub>1</sub> through miniature columns containing 50%, 75%, and 100% Cecil sandy loam showed a decrease in FB<sub>1</sub> elution as soil concentration increased (Figure 3B–D). At 50%, 75%, and 100% Cecil sandy loam, the average concentration of FB<sub>1</sub> in the leachate was approximately 60%, 50%, and 20% of the originally applied FB<sub>1</sub> concentration, respectively, after 30 mL of the solution had passed through the columns (Figure 3B–D). Leaching of Bb through the Cecil sandy loam (Figure 3B–D) columns followed a pattern similar to that seen with pure sand, indicating that Bb did not interact with the soil in the same manner as FB<sub>1</sub>. Nonetheless, both Bb and FB<sub>1</sub> leaching included a rapid elution phase followed by a slowly eluted terminal phase, kinetics consistent with a two-compartment model. The elution profiles of FB<sub>1</sub>, applied to the surface of leachate columns containing 50%, 75%, and 100% Cecil sandy loam (Figure 3B–D) as water extracts of the corn screening, indicated that FB<sub>1</sub> was either chemically altered or more tightly retained by the Cecil sandy loam relative to either washed sand alone (Figure 3A) or Bb (Figure 3A–D). These results are qualitatively similar to those reported using silty clay loam soil (16); however, even at 100% Cecil sandy loam, easily detectable amounts of FB<sub>1</sub> were recovered in the water leachate, indicating the potential for FB<sub>1</sub> to move through the soil and into the groundwater.

To determine if the behavior of FB<sub>1</sub> in a field soil sample would mimic that seen in the miniature columns, FB<sub>1</sub> leaching through PVC columns filled with washed sand was compared to FB<sub>1</sub> leaching through intact field cores ( $n = 3$ ) of Cecil sandy loam soil (Figure 4). Approximately 40% of the applied FB<sub>1</sub> was eluted from the soil cores in the first 10 applications, and after the fifth application, the elution profile appeared to be reaching a steady state. However, FB<sub>1</sub> passed rapidly through the columns filled with sand, and 100% of the FB<sub>1</sub> applied to the columns was recovered in the leachate after 10 water applications (Figure 4).

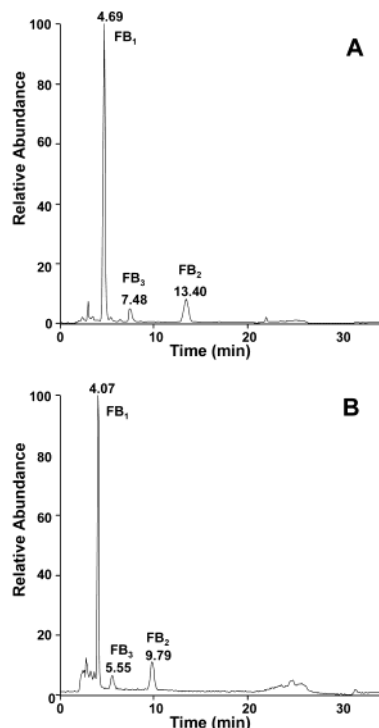
To determine the nature of the interaction between Cecil sandy loam and FB<sub>1</sub>, soil samples were mixed with aqueous solutions of FB<sub>1</sub> and then extracted with various solutions as described in the methods. FB<sub>1</sub> did not bind appreciably to sand (Figure 5A) but was tightly bound by the Cecil sandy loam soil (Figure 5B,C), as evidenced by the fact that very little free FB<sub>1</sub> was detected in the water or acetonitrile/water supernatants (Figure 5B,C). The fact that FB<sub>1</sub> was not extractable from Cecil



**Figure 5.** The ability of soils to bind FB<sub>1</sub>. Briefly, conical tubes (50 mL) were filled with 100% sand (A), 50% sand/50% Cecil sandy loam (B), and 100% Cecil sandy loam (C) and used to test the ability of soils to bind FB<sub>1</sub> and to test the ability of the various extraction solutions to extract any FB<sub>1</sub> bound to the soil. The extraction solutions used were water, 1:1 acetonitrile/water (ACN:H<sub>2</sub>O), 5% formic acid (F), and 1:1 ACN:5% F. Aliquots (30 mL) of an FB<sub>1</sub> water-extracted solution were added to each tube, the tubes were then shaken for 12 h and centrifuged at 240 rcf, and the supernatant was removed. The retention of FB<sub>1</sub> in the soils was determined by comparing the FB<sub>1</sub> samples taken from the supernatant with the FB<sub>1</sub> content of the original solutions. Values are means  $\pm$  SD ( $n = 3$ ), and ND indicates that no FB<sub>1</sub> was detected. One-way analysis of variance indicated that, within each group (low and high), there were significant ( $p < 0.05$ ) differences among extraction solutions for each soil type. Statistical differences among groups were not determined.

sandy loam with acetonitrile/water suggests that the interaction between FB<sub>1</sub> and soil is not hydrophobic. However, extracts using either 5% formic acid or acetonitrile/5% formic acid (**Figure 5B,C**) extracted OPA-positive compounds with the same mobility as pure FB<sub>1</sub>. The presence of fumonisins in the 5% formic acid extract was confirmed on the basis of the MS/MS spectra (data not shown) and single-ion monitoring at  $m/z$  722.3 (FB<sub>1</sub>) and 706.3 (FB<sub>2</sub> and FB<sub>3</sub>) (**Figure 6**).

In a study conducted with silty clay loam, it was concluded that current farm practices should prevent fumonisin from entering groundwater on the basis of the inability to extract FB<sub>1</sub> from soil using 1:1 acetonitrile/water (16). However, worldwide, corn is grown in many different soil types. For example, in the United States, corn is grown commercially in silty clay loam (midwest), sandy loam (southeast), and loamy sand (Delmarva peninsula). These soil types are differentiated texturally by their relative percentage of clay, sand, and silt content, with the silty clay loam having the highest percentage of clay and the lowest percentage of sand, and loamy sand having the least clay and the most sand (18). The results of the present study using Cecil sandy loam soil indicate that FB<sub>1</sub> is quite stable in the soil environment and suggests that, while a portion may be tightly bound, under certain environmental conditions the FB<sub>1</sub> could be released and become biologically available. If FB<sub>1</sub> is mobilized by rainwater, then it could enter and move through both simple and more complex soils and be recovered chemically intact in the leachate. Therefore, it is possible that FB<sub>1</sub> could alter the biological activity in the soil flora and fauna, and it is also possible that FB<sub>1</sub> from corn debris in field situations could enter the groundwater. Also, the more complex the soil, the more likely it is that FB<sub>1</sub> will be retained in the



**Figure 6.** HPLC chromatogram of electrospray single-ion monitoring MS for (A) water extracts of corn screening ( $m/z$  FB<sub>1</sub> = 722.3 at 4.69 min and  $m/z$  FB<sub>2</sub> and FB<sub>3</sub> = 706.3 at 13.4 and 7.48 min, respectively) and (B) a single 5% formic acid extract of 100% Cecil sandy loam that had been treated as described in **Figure 5** ( $m/z$  FB<sub>1</sub> = 722.3 at 4.07 min and  $m/z$  FB<sub>2</sub> and FB<sub>3</sub> = 706.3 at 9.79 and 5.55 min, respectively). The MS/MS mass spectra were confirmed on the basis of comparison with authentic standards of FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> (provided by Ronald Plattner, USDA/ARS, Peoria, IL) (data not shown).

soil matrix, although biological availability remains unknown. Acid conditions could also facilitate the mobilization of FB<sub>1</sub> bound presumably via ionic interactions with soil constituents.

## SAFETY

Fumonisin B<sub>1</sub> is a known liver and kidney carcinogen in rodents; therefore, it should be handled using proper precautionary measures.

## ABBREVIATION

FB<sub>1</sub>, fumonisin B<sub>1</sub>; Bb, bromophenol blue.

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